Single and Multiple In-Season Measurements as Indicators of At-Harvest Cotton Boll Damage Caused by Verde Plant Bug (Hemiptera: Miridae)

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ABSTRACT The ability to monitor verde plant bug, *Creontiades signatus* Distant (Hemiptera: Miridae), and the progression of cotton, *Gossypium hirsutum* L., boll responses to feeding and associated cotton boll rot provided opportunity to assess if single in-season measurements had value in evaluating at-harvest damage to bolls and if multiple in-season measurements enhanced their combined use. One in-season verde plant bug density measurement, three in-season plant injury measurements, and two at-harvest damage measurements were taken in 15 cotton fields in South Texas, 2010. Linear regression selected two measurements as potentially useful indicators of at-harvest damage: verde plant bug density (adjusted $r^2 = 0.68$; $P = 0.0004$) and internal boll injury of the carpel wall (adjusted $r^2 = 0.72$; $P = 0.004$). Considering use of multiple measurements, a stepwise multiple regression of the four in-season measurements selected a univariate model (verde plant bug density) using a 0.15 selection criterion (adjusted $r^2 = 0.74$; $P = 0.0002$) and a bivariate model (verde plant bug density–internal boll injury) using a 0.25 selection criterion (adjusted $r^2 = 0.76$; $P = 0.0007$) as indicators of at-harvest damage. In a validation using cultivar and water regime treatments experiencing low verde plant bug pressure in 2011 and 2012, the bivariate model performed better than models using verde plant bug density or internal boll injury separately. Overall, verde plant bug damaging cotton bolls exemplified the benefits of using multiple in-season measurements in pest monitoring programs, under the challenging situation when at-harvest damage results from a sequence of plant responses initiated by in-season insect feeding.

KEY WORDS sucking bugs, plant bugs, pest monitoring, cotton boll damage

A fundamental need in implementing pest management monitoring programs is the availability of in-season measurements that can be used as indicators of economic damage at harvest (i.e., the measurements can reliably represent the relationship of economic damage to pest activity; Pedigo et al. 1986). Measurements of pest activity as in-season indicators include insects per plant and percentage infested plants. Plant responses to feeding also can serve as in-season indicators, including defoliation and localized plant and fruit wounding. In-season insect and plant measurements may provide acceptable indication of economic damage if the pest causes obvious and determinant loss to the harvestable portion of the plant. For field crops, in-season measurements must serve as indicators of economic damage that commonly expresses itself later in plant development, such as when leaf damage reduces seed yield on grain crops. Examples of this approach in many cropping systems are presented by Pedigo and Buntin (1994) and specifically for cotton by Benedict et al. (1989).

Applied to cotton insect pest management, selection of an in-season measurement is based on its reliability as an indicator of at-harvest damage and operational factors such as sampling efficiency (e.g., Parajulee et al. 2006, Toews et al. 2009, Brewer et al. 2012a). The use of more than one in-season measurement as an indicator of at-harvest damage may improve the reliability of predicting subsequent damage, and this benefit may off-set any increased operational cost in taking multiple measurements. This concept is especially relevant when at-harvest damage is dependent upon a sequence of plant responses triggered by insect feeding. This scenario is applicable to the case of sucking bugs (Hemiptera: Pentatomidae and Miridae) that feed on green (immature) bolls of cotton, *Gossypium hirsutum* L., and may introduce cotton boll rot microorganisms during feeding (Medrano et al. 2007, 2009). A sequence of plant and associated disease responses are triggered that may lead to economic damage of lint and seed of open (mature) bolls at harvest (Greene et al. 2001, Armstrong et al. 2010).

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The sequence of events initiated by sucking bug feeding on cotton bolls has been documented for the southern green stink bug, *Nezara viridula* L. (Hemiptera: Pentatomidae), (Medrano et al. 2007, 2009), and is similar for verde plant bug, *Creontiades signatus* Distant (Hemiptera: Miridae), based on assessment of plant response to verde plant bug feeding and associated cotton boll rot (Armstrong et al. 2010, Brewer et al. 2012b). Darkened lesions on the external carpel wall result from stylet probing. If the stylets penetrate into the soft developing lint and seed tissue, the point of stylet piercing through the inner carpel wall may be visible when the boll is opened, and callus tissue on the inner wall may occur around the penetration point. Injury to lint and seed within individual locules may occur. Lint staining is caused by enzymatic activity of the saliva followed by tissue degradation, and seed injury is caused from direct stylet penetration of seeds within reach of stylets penetrating the carpal wall. Further lint and seed degradation occurs if pathogens are introduced which cause cotton boll rot (Medrano et al. 2007). Lint staining, seed deterioration and cotton boll rot development progresses over several weeks, often remaining localized within the locules where stylet penetration occurs. When green bolls are opened, visibility of symptoms will depend on the severity and progression of injury from insects and associated disease. The distinction between boll damage caused by insect feeding and disease becomes more apparent as the disease progresses (Medrano et al. 2007, 2009). Damage to the open boll varies at harvest, depending on the severity, intensity, and timing of events including the number of stylet probes, extent of pathogen introduction, and the number of locules fed upon. Boll rot fully expresses itself at harvest in open bolls, and its presence is typically distinguishable from damage caused only by direct insect injury (Medrano et al. 2007, 2009). Boll damage ranges from negligible with some indications of feeding on the carpel wall, internal locule-specific damage with no cotton boll rot, locule-specific damage with cotton boll rot, and more extensive damage and cotton boll rot in multiple locules (Medrano et al. 2009).

For verde plant bug in particular, bacteria have been isolated from green bolls with visible symptoms of cotton boll rot and that were exposed to verde plant bug feeding in a controlled setting (Brewer et al. 2012b). Cotton boll rot is a major component of overall open boll damage in commercial cotton fields where verde plant bug occurs (Brewer et al. 2012b).

For pest management purposes, boll protection guidelines for stink bugs have been based on insect density or frequency of green bolls with internal damage (Greene et al. 2001; Reay–Jones et al. 2009, 2010). Here we consider the verde plant bug, which has become a threat to cotton in South Texas during the last decade (Coleman 2007). Using field-based measurements selected from the sequence of events discussed above, we consider whether a single measurement has value in indicating subsequent at-harvest boll damage and if multiple measurements enhance their combined use as indicators of boll damage.

Materials and Methods

Insect and Plant Response Measurements. Several in-season insect and plant response measurements were chosen as potential indicators of at-harvest boll damage. Insect data were collected during mid-bloom (about week 3 to 4 of bloom) from 15 cotton fields in the coastal cotton growing region of South Texas, 2010. Green boll data were taken 2 wk later. Fields were randomly selected from a list provided by crop consultants, with the exception that eight were selected within 8 km of the nearest coastline, inland bay, or coastal waterway (designated as coastal fields) and seven further inland (designated as inland fields). Locations by county were three in Calhoun County, one in Aransas County, six in Nueces County, three in Kleberg County, and two in Cameron County. Verde plant bug was the dominant boll-feeding sucking bug, and cotton boll rot was detected (Brewer et al. 2012b). Insecticide use was restricted to early season prefloral bud (square) protection and occurred before week 1 of bloom.

The in-season insect measurement was verde plant bug density at mid-bloom estimated using the beat bucket sampling method (n = 120–200 plants per field). This method provided acceptable (low variability, time efficient, and low cost) estimates of verde plant bug densities (Brewer et al. 2012a). The plant measurements taken from randomly selected green bolls 1.0–2.7 cm in diameter (n = 120–150 bolls per field) were external and internal boll injury caused by stylet penetration, and internal symptoms of cotton boll rot. Bolls in this size range were known to be readily fed upon and damaged by verde plant bug (Armstrong et al. 2013). They were inspected externally for darkened lesions characteristic of stylet probing. The bolls were then opened and the inner carpel wall was inspected for callous tissue and stylet penetration points (Toews et al. 2009). Soft lint and seed tissue were inspected for apparent symptoms of cotton boll rot (Medrano et al. 2009).

These in-season data were matched with subsequent open boll damage at harvest. Lint and seed was thoroughly inspected on randomly selected open bolls in the field (n = 120–150 bolls per field). Damage was scored using a five class locule damage scale. The scale ranged from 0 representing no damage detected; 1, 2, and 3 representing a progression of damage from localized damage in one locule to damage in most locules; and four representing damage in all locules (Lei et al. 2003). The scale was implemented for visual field assessment by equating the number of damaged locules to the same scalar, assuming damage affected at least a quarter of the locule. The open bolls also were inspected for cotton boll rot symptoms (Medrano et al. 2007).

Assessing Variable Relevance with Linear Regression. The mean number of verde plant bug per plant was calculated for each field. Plant response data on green bolls were used to calculate proportions of bolls with external injury of the carpel wall, bolls with internal injury of the carpel wall, and bolls with symptoms of cotton boll rot for each field. Using the open boll data, mean damage score (0–4 scale) and proportion of open bolls with cotton boll rot were calculated for each field. The damage score (dependent variable) was selected for use as a composite at-harvest damage index based on its regression on proportion of open bolls with cotton boll rot (independent variable) (see Results). Separate linear regressions of damage score on the four in-season measurements (independent variables) were estimated in an initial assessment of variables as indicators of at-harvest damage. A qualitative indicator variable also was included to distinguish coastal fields from inland fields. It was used to test the hypothesis of a common linear regression for coastal and inland fields (α was set at 0.10, which is common for regression line comparisons) (Neter et al. 1985, Freund and Littell 2000). No pattern of deviation from linear regression assumptions was seen inspecting residual plots.

Selection of Single or Multiple In-Season Measurements. The four independent variables were entered into stepwise multiple regression using 0.15 and 0.25 variable selection significance levels to explore sensitivity of accepting variables in a multiple regression model (Freund and Littell 2000) and in view of biological and operational considerations. Damage score was the dependent variable. The four independent variables were the same as above: verde plant bug per plant (x_1), proportion of green bolls with external injury of the carpel wall (x_2), proportion of green bolls with internal injury of the carpel wall (x_3), and proportion of green bolls with internal symptoms of boll rot (x_4). Residual plots showed no pattern of deviation from regression assumptions. A qualitative indicator variable was not included (see Results).

Model Validation. A univariate verde plant bug density model, a univariate internal boll injury model, and a bivariate verde plant bug density–internal boll injury model (see Results for selection rationale) were compared in a validation exercise using external data to help identify a generalized model applicable to a range of conditions (e.g., Vernier et al. 2008). Data for model validation came from a water regime–cultivar experiment conducted in 2011 and 2012, Corpus Christi, TX. This site was coastal based on the criteria used in this study. Treatments were arranged in a split plot design with five replications. Three water regimes were set as the main plot and three (2011) or two (2012) cultivars were set as the split plot. Each subplot where data were taken measured 30 m (2011) or 60 m (2012) in length by four rows on 96.5 cm centers. The three water regimes were dryland, irrigation scheduled at 75% of evapotranspiration replacement, and irrigation scheduled at 90% of evapotranspiration replacement. The cultivars were Phytogen 367 WRF (PhytoGen Seed, Dow AgroSciences, Indianapolis, IN), Deltapine 1032 B2RF (Deltapine, Monsanto, St. Louis, MO [used in 2011 only]), and Stoneville 5458 B2RF (Bayer CropScience, Research Triangle Park, NC). Agronomic practices were standard, and no insecticides were used.

Verde plant bug arrived 4 wk after first bloom. Data were taken on the inner two rows, with half the plot devoted to insect and boll sampling and half the plot left undisturbed for yield. Insect counts of nymphs and adults were based on a 10-plant beat bucket sample per plot. Counts were adjusted to a plant basis, and averaged across the five replications of each of the nine (2011) and six (2012) water regime–cultivar treatment combinations (n = 50 plants per treatment combination). Two weeks later, the internal injury of the carpel wall measurement was taken by inspecting 15 green bolls in the same plots (n = 75 plants per treatment combination). Boll damage scores were taken at harvest on the same number of open bolls. Seed cotton was removed from the undisturbed portion of each plot using a two row cotton picker (John Deere, Moline, IL). It was weighed, and a representative sample (300–400 g) was ginned using a 10 saw laboratory cotton gin (Continental Eagle Corp., Prattville, AL). Percent lint and seed weight of the sample was used to calculate lint and seed yield on a pounds per acre basis (1,000 lbs/acre = 1,140 kg/ha).

The observed values (i.e., verde plant bug density and proportion of bolls with internal injury of the carpel wall) of the water regime–cultivar treatment combinations were used as input variables in the three selected models to obtain prediction of the boll damage score for each treatment combination. The observed boll damage scores were regressed on the predicted scores for each of the models across the 2011 and 2012 experiments (n = 15 for each regression). To test validity of the models, the hypotheses of a linear relationship and slope = 1 were tested (Neter et al. 1985). In addition, mean lint and seed weights of the cultivar–water regime treatments also were individually regressed on means of at-harvest boll damage score, verde plant bug density, and proportion of internal injury of the carpel wall (six regressions for each year) to further assess damage under the conditions experienced in 2011 and 2012.

Results and Discussion

Verde plant bug averaged 0.42 bugs per plant using the beat bucket sampling method during 2010, and represented ≈99% of the boll-feeding sucking bugs collected in the region (Brewer et al. 2012a). The range of verde plant bug activity and boll damage was representative of that experienced when verde plant bug became recognized as a threat to cotton in the region (Coleman 2007, Armstrong et al. 2010). Cotton boll rot and damage to open bolls was subsequently detected near harvest in coastal fields where verde plant bug was detected and yield losses were observed (up to 25% of open bolls with cotton boll rot and damage score of 1.5). Cotton boll rot was a major contributor to overall damage of open bolls in these fields ($y = 5.12x + 0.21$; adjusted $r^2 = 0.94$; $F = 156$; df = 2, 11; $P = 0.0001$) (Brewer et al. 2012b). Based on these findings, the damage score was selected as a

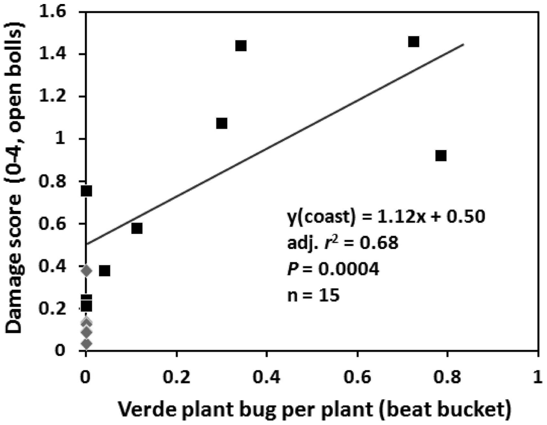

Fig. 1. Regression of mean damage score of open bolls at harvest on mean number of verde plant bug per plant detected mid-bloom with a beat bucket. Symbols indicate coastal fields within 8 km of the coastline and inland bays (squares) and inland fields (diamonds), South Texas, 2010. A linear regression was estimated for coastal fields (coast).

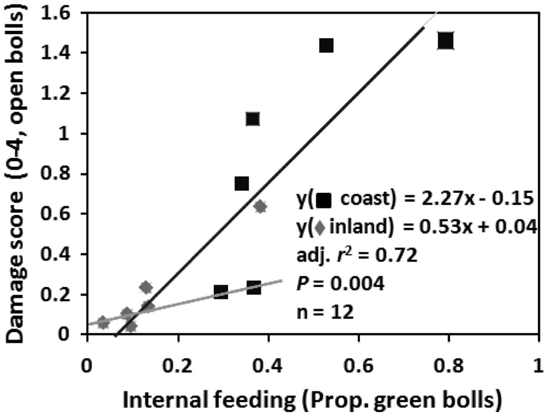

Fig. 2. Regression of mean damage score of open bolls at harvest on mean proportion of green bolls with internal injury to the carpel wall. Symbols indicate coastal fields within 8 km of the coastline and inland bays (squares) and fields further inland (diamonds), South Texas, 2010. Linear regressions were estimated for coastal (coast) and inland (inland) fields.

composite at-harvest damage index and used as the dependent variable in further regression analyses.

Assessing Variable Relevance with Linear Regression. Mean damage score of open bolls at harvest was linearly related to mean verde plant bug density observed mid-bloom (adjusted $r^2 = 0.68$; $F = 16.1$; df = 2, 12; $P = 0.0004$). No verde plant bugs were detected in inland fields; therefore, a regression was estimated for coastal fields only (Fig. 1).

For the in-season plant measurements, green bolls with external injury of the carpel wall (darkened lesions) were very common in all fields (>70% of bolls). However, the proportion of green bolls with external injury was not indicative of the mean damage score at harvest (linear regression: adjusted $r^2 = 0.31$, $P = 0.12$), and there was no indication of differences in the external injury–damage score relationship between coastal and inland fields ($P = 0.78$). The proportion of green bolls with internal symptoms of cotton boll rot provided little indication of at-harvest boll damage as well (linear regression: adjusted $r^2 = 0.32$, $P = 0.09$), and the slopes of the coastal and inland field regressions did not differ ($P = 0.80$). In contrast, proportion of green bolls with internal injury of the carpel wall was a good indicator of damage score (linear regression: adjusted $r^2 = 0.72$, $F = 10.3$; df = 3, 8; $P = 0.004$). The slopes of the coastal and inland field regressions differed at $\alpha = 0.10$ ($t = -1.95$; df = 1, 8; $P = 0.09$) (Fig. 2). The relatively flat slope and low damage scores for the inland field regression were consistent with verde plant bug not being detected at inland fields (Fig. 1).

In comparison, Toews et al. (2009) explored use of boll injury as an indicator of damage and found better fidelity of external boll injury to boll damage caused by stink bugs than reported here. However, they also reported the internal boll injury measurement provided a better relationship to boll damage as consistent with our findings for verde plant bug. Interestingly, the relationship of in-season internal symptoms of cotton boll rot in green bolls to at-harvest boll damage was not useful, even though this relationship represented a further step along the sequence of plant responses initiated by insect feeding on bolls. Disease symptoms progress significantly over the course of several weeks (Medrano et al. 2009); therefore, visual in-season evaluation of disease in green bolls may be prone to error.

Selection of Single or Multiple In-Season Measurements. As noted above, differences between coastal and inland fields were modest for the internal boll injury–damage score regression ($P = 0.09$ for testing differences in slopes, Fig. 2) or not relevant for the verde plant bug density–damage score regression (i.e., verde plant bug was not at detectable levels in inland fields, Fig. 1); therefore, the qualitative indicator variable was not considered in exploring use of multiple measurements in a stepwise multiple regression. The four independent variables were maintained as initial variable entries in the multiple regression, but the two in-season measurements identified with linear regression were expected to be highlighted.

The stepwise multiple regression selected a univariate model (verde plant bug density) using the standard 0.15 selection criterion ($y = 2.06x_1 + 0.27$; adjusted $r^2 = 0.74$; $F = 32.3$; df = 1, 10; $P = 0.0002$). Adjusting the selection criterion to 0.25, the variable proportion of bolls with internal injury of the carpel wall was added ($y = 1.58x_1 + 0.65x_3 + 0.11$; adjusted $r^2 = 0.76$; $F = 18.3$; df = 2, 9; $P = 0.0007$), although model r^2 value increased modestly from the univariate model. The other independent variables were not chosen in the selection process.

These results suggested similar benefits of the two models identified using stepwise multiple regression. It was intriguing that the univariate verde plant bug density model performed well as an indicator of at-harvest damage. Operationally, it was much easier to

Table 1. Validation of a univariate verde plant bug density model (selected with the 0.15 multiple regression selection criteria), a univariate internal boll injury model (selected from linear regression), and a bivariate verde plant bug density–internal boll injury model (selected using a 0.25 multiple regression selection criteria) as indicators of at-harvest cotton boll damage, Corpus Christi, TX, 2011 and 2012

Cultivar/water[a]	Lint wt.[b]	Seed wt.[b]	Verde plant bug obs.[c]	Boll injury obs.[c]	Damage score obs.[c]	Damage pred. verde[d]	Damage pred. boll[d]	Damage pred. verde-boll[d]
2011								
Phytogen/dry	643 ± 21	856 ± 21	0.04 ± 0.02	0.31 ± 0.06	0.040 ± 0.016	0.351	0.472	0.372
Phytogen/low	771 ± 52	1,034 ± 61	0.05 ± 0.03	0.33 ± 0.04	0.067 ± 0.021	0.372	0.507	0.406
Phytogen/high	919 ± 20	1,202 ± 24	0.14 ± 0.09	0.29 ± 0.05	0.120 ± 0.033	0.556	0.437	0.522
Deltapine/dry	569 ± 44	735 ± 44	0.00 ± 0.00	0.41 ± 0.07	0.067 ± 0.030	0.269	0.648	0.377
Deltapine/low	716 ± 55	954 ± 70	0.02 ± 0.02	0.30 ± 0.08	0.107 ± 0.021	0.303	0.454	0.333
Deltapine/high	867 ± 48	1,113 ± 49	0.04 ± 0.02	0.20 ± 0.02	0.067 ± 0.021	0.351	0.278	0.303
Stoneville/dry	595 ± 29	829 ± 29	0.02 ± 0.02	0.25 ± 0.05	0.040 ± 0.027	0.310	0.366	0.305
Stoneville/low	768 ± 14	1,091 ± 20	0.04 ± 0.02	0.37 ± 0.03	0.080 ± 0.013	0.351	0.578	0.416
Stoneville/high	801 ± 31	1,130 ± 31	0.02 ± 0.02	0.29 ± 0.08	0.067 ± 0.021	0.310	0.437	0.289
2012								
Phytogen/dry	560 ± 76	766 ± 93	0.01 ± 0.01	0.67 ± 0.06	0.314 ± 0.105	0.289	1.105	0.559
Phytogen/low	658 ± 52	898 ± 62	0.04 ± 0.02	0.71 ± 0.08	0.510 ± 0.088	0.351	1.176	0.634
Phytogen/high	668 ± 56	910 ± 72	0.07 ± 0.03	0.72 ± 0.10	0.542 ± 0.084	0.413	1.193	0.689
Stoneville/dry	552 ± 57	801 ± 74	0.10 ± 0.05	0.89 ± 0.05	0.350 ± 0.037	0.474	1.492	0.849
Stoneville/low	621 ± 38	891 ± 49	0.19 ± 0.08	0.81 ± 0.04	0.500 ± 0.095	0.659	1.352	0.939
Stoneville/high	713 ± 74	1,045 ± 112	0.04 ± 0.03	0.79 ± 0.07	0.457 ± 0.124	0.351	1.316	0.685

[a] The cultivars were Phytogen 367 WRF, Deltapine 1032 B2RF (used in 2011 only), and Stoneville 5458 B2RF. The three water regimes were dryland (dry), irrigation scheduled at 75% of evapotranspiration replacement (low), and irrigation scheduled at 90% of evapotranspiration replacement (high).

[b] Measured in lbs per acre ± SEM (1,000 lbs per acre = 1,140 kg/ha).

[c] Observed measurements (±SEM) were verde plant bug per plant at mid-bloom (verde plant bug obs.), proportion of green bolls with internal injury 2 wk later (boll injury obs.), and at-harvest open boll damage (0–4 scale; Lei et al. 2003) (damage score obs).

[d] Predicted damage scores from the univariate verde plant bug density model (damage pred. verde), univariate internal boll injury model (damage pred. boll), and bivariate verde plant bug density–internal boll damage model (damage pred. verde-boll). See text for results of linear regression of observed and predicted values.

only estimate verde plant bug density using the beat bucket method, in contrast to adding the process of opening green bolls to estimate proportion of bolls with internal injury to the carpel wall. Biologically, adding an internal boll injury measurement provided opportunity to confirm another step further along the sequence of plant responses initiated by insect feeding (Medrano et al. 2009). Based on our findings, either of the models may be acceptable, and field performance may depend on a combination of biological, operational, as well as statistical considerations. Therefore, the univariate verde plant bug density model and bivariate verde plant bug density–internal boll injury model were entered into the validation exercise. A univariate internal boll injury model was included based on its similarities to the univariate verde plant bug density model using linear regression (Figs. 1 and 2).

Model Validation. There was low damage detected in the validation across all treatment combinations across both years (range, of 0.04–0.54 for damage score means [Table 1] compared with a high score of 1.5 in 2010 [Fig. 1]). Relatively low verde plant bug pressure was also seen (<0.20 verde plant bug per plant) compared with up to 0.79 verde plant bug per plant in 2010. A persistent drought in 2011 and 2012 was also notable (i.e., <8 cm and <16 cm of rainfall 1 April through 30 August, respectively, compared with 45.7 cm in 2010 and a 35.5 cm average over 125 yr; National Weather Service 2012). The predicted damage score means varied considerably dependent upon the model used (Table 1). The predicted damage scores of the univariate verde plant bug model deviated from the observed values as shown by poor linear fit ($P = 0.12$), In contrast, the predicted values from the univariate internal boll injury model were linearly related to observed values ($F = 65.2$; df = 1, 13; $P < 0.0001$), but the model overestimated at-harvest damage (slope of 0.42 differed significantly from 1.0, $F = 116$; df = 1, 13; $P < 0.0001$) (Table 1). This observed overestimation was particularly problematic because predicted damage scores approached 1.5 that were associated with substantial cotton boll rot and yield loss observations in 2010 (Brewer et al. 2012b). The matching observed damage scores in the validation never exceeded 0.55 (Table 1). Performance improved considerably when using both in-season measurements in the bivariate model, as judged by a significant linear regression of the observed values on the predicted values ($F = 36.7$; df = 1, 13; $P < 0.0001$) and no significant deviation of the estimated regression slope of 0.85 from 1.0 ($P = 0.29$).

Lint and seed yield of the cultivar–water regime treatment combinations varied, with the dryland treatments producing relatively low yields and the 90% evapotranspiration irrigation treatments producing higher yields (Table 1), compared with historical records (1972–2004) of ≈650–700 lbs of lint per acre for this dryland growing region (Chen and Miranda 2008). In this low verde plant bug density and drought situation, the water regime effect likely overshadowed any association of lint and seed yield on the independent variables of at-harvest boll damage score, verde plant bug density, and proportion of internal injury of the carpel wall. Only one regression in 2011 detected a modest linear relationship (verde plant bug density-lint weight regression, adjusted $r^2 = 0.43$, $P = 0.03$), and none of the regressions were significant in 2012 ($P > 0.07$). The lack of relationship of the variables to yield was disappointing from an economic injury level viewpoint, but did not negate the benefits in using the two in-season measurements as indicators of at-harvest boll damage in our recent years of low verde plant bug pressure. In cases where environmental fluctuations may affect the populations of interest, the value and challenge of using external validation data sets to help identify generalized models for field application becomes apparent (e.g., Vernier et al. 2008). Our validation is such an example where extended drought conditions likely affected cotton yield and possibly depressed verde plant bug populations as well as other insects. The bivariate model outperformed the others, although continuing validation or manipulating verde plant bug pressure experimentally would be helpful to consider model performance above the range of low verde plant bug pressure experienced in 2011 and 2012.

In regard to operational considerations in selecting a model for field use, the insect monitoring technique was readily implemented (Brewer et al. 2012a), and there was an obvious cost in adding an internal boll injury measurement (i.e., opening green bolls in the field, Toews et al. 2009). Reay–Jones (2009) developed protocols for sampling stink bugs, but concluded that sampling bolls and inspecting for internal boll injury was a more reliable indicator of damage despite the added operational cost, likely because of the temporal presence of stink bugs and the relative persistence of boll injury (Reay–Jones et al. 2010). The 2 yr validation in our study was consistent with this viewpoint that use of in-season insect density alone was problematic. The validation also added evidence that combining use of verde plant bug density and internal boll injury estimation improved their usefulness as indicators of at-harvest damage and avoided overestimation of boll damage (Table 1). More generally, the case of verde plant bug damaging cotton bolls exemplified the benefits of using multiple in-season measurements under the challenging situation of evaluating at-harvest damage resulting from a sequence of plant responses initiated by in-season insect feeding.

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References Cited

Armstrong, J. S., M. J. Brewer, R. D. Parker, and J. J. Adamczyk, Jr. 2013. Verde plant bug (Hemiptera: Miridae) feeding injury to cotton bolls characterized by boll age, size, and \damage ratings. J. Econ. Entomol. 106: 189–195.

Armstrong, J. S., R. J. Coleman, and B. L. Duggan. 2010. Actual and simulated injury of *Creontiades signatus* Distant (Hemiptera: Miridae) feeding on cotton bolls. J. Entomol. Sci. 45: 170–177.

Benedict, J. H., K. M. El-Zik, L. R. Oliver, P. A. Roberts, and L. T. Wilson. 1989. Economic injury levels and thresholds of pests of cotton, pp. 121–151. *In* R. E. Frisbie, K. M. El-Zik, and L. T. Wilson (eds.), Integrated Pest Management Systems and Cotton Production. Wiley, Somerset, NJ.

Brewer, M. J., D. J. Anderson, J. S. Armstrong, and R. T. Villanueva. 2012a. Sampling strategies for square and boll-feeding plant bugs (Hemiptera: Miridae) occurring on cotton. J. Econ. Entomol. 105: 896–905.

Brewer, M. J., J. A. Armstrong, E. G. Medrano, and J. F. Esquivel. 2012b. Association of verde plant bug, *Creontiades signatus* (Hemiptera: Miridae), with cotton boll rot. J. Cotton Sci. 16: 1–8.

Chen, S., and M. J. Miranda. 2008. Modeling Texas dryland cotton yields, with application to crop insurance actuarial rating. J. Agric. Appl. Econ. 40: 239–252.

Coleman, R. J. 2007. *Creontiades signatus:* a plant bug pest of cotton in South Texas, pp. 38–41. *In* Proceedings, Beltwide Cotton Conferences, New Orleans, LA, 8–11 January 2007. Natl. Cotton Counc. Am., Memphis, TN.

Freund, R. J., and R. C. Littell. 2000. SAS system for regression, 3rd ed. SAS Institute, Cary, NC.

Greene, J. K., S. G. Turnipseed, M. J. Sullivan, and O. L. May. 2001. Treatment thresholds for stink bugs (Hemiptera: Pentatomidae) in cotton. J. Econ. Entomol. 94: 403–409.

Lei, T., M. Khan, and L. Wilson. 2003. Boll damage by sucking pests: an emerging threat, but what do we know about it?, pp. 1338–1344. *In* Proceedings, World Cotton Research Conf. 3, Cape Town, Rep. South Africa.

Medrano, E. G., J. F. Esquivel, and A. A. Bell. 2007. Transmission of cotton seed and boll rotting bacteria by the southern green stink bug (*Nezara viridula* L.). J. Appl. Microbiol. 103: 436–444.

Medrano, E. G., J. F. Esquivel, R. L. Nichols, and A. A. Bell. 2009. Temporal analysis of cotton boll symptoms resulting from southern green stink bug feeding and transmission of a bacterial pathogen. J. Econ. Entomol. 102: 36–42.

National Weather Service. 2012. South Texas climate normals, records & rankings. National Weather Service Weather. (www.srh.noaa.gov/crp/).

Neter, J., W. Wasserman, and M. H. Kutner. 1985. Applied linear statistical models: regression, analysis of variance, and experimental designs, 2nd ed. Richard D. Irwin, Homewood, IL.

Parajulee, M. N., R. B. Shrestha, and J. F. Leser. 2006. Sampling methods, dispersion patterns, and fixed precision sequential sampling plans for western flower thrips (Thysanoptera: Thripidae) and cotton fleahoppers (Hemiptera: Miridae) in cotton. J. Econ. Entomol. 99: 568–577.

Pedigo, L. P., and G. D. Buntin. 1994. Handbook of sampling methods for arthropods in agriculture. CRC, Boca Raton, FL.

Pedigo, L. P., S. H. Hutchins, and L. G. Higley. 1986. Economic injury levels in theory and practice. Annu. Rev. Entomol. 31: 341–368.

Reay–Jones, F.P.F., J. K. Greene, M. D. Toews, and R. B. Reeves. 2009. Sampling stink bugs (Hemiptera: Pentatomidae) for population estimation and pest management in southeastern cotton production. J. Econ. Entomol. 102: 2360–2370.

Reay–Jones, F.P.F., M. D. Toews, J. K. Greene, and R. B. Reeves. 2010. Development of sampling plans for cotton bolls injured by stink bugs (Hemiptera: Pentatomidae). J. Econ. Entomol. 103: 525–532.

Toews, M. D., E. L. Blinka, J. W. Van Duyn, D. A. Herbert, Jr., J. S. Bacheler, P. M. Roberts, and J. K. Greene. 2009. Fidelity of external boll feeding lesions to internal damage for assessing stink bug damage in cotton. J. Econ. Entomol. 102: 1344–1351.

Vernier, P. R., F.K.A. Schmiegelow, S. Hannon, and S. G. Cumming. 2008. Generalizability of songbird habitat models in boreal mixedwood forests of Alberta. Ecol. Modelling 211: 191–201.

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